REMARKS

Claims 1-25 are pending. Claims 20-25 are presently withdrawn from consideration. Claims 1-19 stand rejected in this application. Claim 11 has been canceled. New claims 26-31 have been added. Support for the new claims can be found throughout the specification, or the claims as originally filed. No new matter has been added.

Amendment or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to more particularly point out and distinctly claim the invention to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

The Invention

The present application discloses and claims method of treating neurodegenerative disease by delivering a vector that comprises a *nucleic acid sequence encoding glutamic acid decarboxylase (GAD)* to *target cells of the central nervous system* (e.g., a region of the brain), to treat or reduce a neurodegenerative disease. Applicants have discovered that increased levels of GAD can ameliorate neurodegenerative diseases, and that gene therapy can be used effectively to increase GAD in the central nervous system.

Claim Objections

Claims 1-11 are objected to because they encompass non-elected subject matter by claiming diseases other than Parkinson's disease. Applicants reiterate their position that claim 1 is a *generic* claim spanning various species of neurodegenerative disease identified in the restriction requirement and, as such, amendment to recite only the elected species is unnecessary. The Examiner's attention is directed to § 806.04(a)-(e) of the Manual of Patent Examining Procedure.

Rejection of Claims 1-19 Under 35 U.S.C. §112 First Paragraph

Claims 1-19 have been rejected under 35 U.S.C. § 112, first paragraph. In particular, the Office Action asserts that:

> [t]he specification, *while being enabling for a method of treating Parkinson's disease by administering to the subthalmic nucleus (STN) and rAAV vector comprising a nucleotide sequence encoding glutamic acid decarboxylase (GAD)*, wherein the symptom of Parkinson's disease is ameliorated, *does not reasonably provide enablement for the use of any type of vector* for the treatment of Parkinson's disease, *nor any target tissue other than the STN*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims (emphasis added).

As supporting this ground for rejection, the Office Action cites certain references suggesting that gene therapy is an unpredictable art, and as such Applicants should only be entitled to delivery using the AAV vectors, and delivery only to the STN.

Applicants respectfully disagree and traverse the rejection. Once Applicant's teachings with respect to GAD expression are known, the design of particular vectors and the selection of various CNS sites is clearly within the capabilities of one skilled in the art.

It is well established that enablement is not precluded by the need for experimentation, even a large quantity of experimentation, if the specification, in combination with the knowledge available in the art, provides guidance regarding how to carry out the experimentation such that the experimentation is not "undue." *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404 (citing *In re Angstadt*, 537 F.2d 489, 502-504, 190 USPQ 214, 218 (CCPA 1976)).

Furthermore, "(i)The law is clear that patent documents need not include subject matter that is known in the field of the invention and is in the prior art, for patents are written for persons experienced in the field of the invention *See Viviv Technologies, Inc. v. American Science and Engineering, Inc.* 200 F.3d 795, 804, 53 USPQ2d 1289, 1295 (Fed. Cir. 1999) ('Patents are written by and for the skilled artisans')." (2) "To hold otherwise would require

every patent document to include a technical treatise for the unskilled reader. Although an accommodation to the 'common experience' of lay persons may be feasible, it is an unnecessary burden for inventors and has long been rejected as a requirement for patent disclosures. *See Atmel Corp.*, 198 F.3d at 1382, 53 USPQ2d at 1230 (Fed. Cir. 1999).

The Examiner is attempting to use this rejection to limit the scope of Applicant's claims to cover only the embodiment of the invention that is disclosed in the working examples. The specification of the present invention however provides adequate teaching and guidance to enable one of ordinary skill in the art to make and use the claimed methods of the present invention to treat neurodegenerative diseases using vectors carrying the GAD gene, and delivering these vectors to a region of the central nervous system that requires modification with the GAD gene. The claims clearly recite methods that are sufficiently enabled by the specification of the present invention. Accordingly, Applicant is entitled to a claim coverage for all subject matter that one of ordinary skill in the art would gather from the teachings and guidance of Applicant's specification, as well as from the knowledge available in the art.

The working examples provided by the specification of the present invention are *merely illustrative* of the underlying inventive concept of Applicant's invention – they do *not* represent the sum total of Applicant's underlying inventive concept. Accordingly, the scope of Applicant's claimed methods should not be limited to only the use of the AAV vector or delivery to only the subthalmic nucleus, because Applicant has provided adequate disclosure for other suitable vectors that can be readily substituted into the methods disclosed by the present specification to deliver the GAD gene to any region of the brain, not just the subthalmic nucleus. Moreover, the working example demonstrates the proof-of-principle that GAD overexpression in a region of the brain associated with a neurodegenerative disease, such as Parkinson's disease, can help ameliorate the disease. The same methodology described in the application can readily be applied to other neurodegenerative diseases that require modification with GAD.

There are at least four reasons why Applicant's claims are fully enabled commensurate with the scope of the claimed invention:

(A) Knowledge available in the art

At the time the invention was filed (priority application was filed in May 2000), the knowledge available to the skilled artisan, was well established for treating neurodegenerative diseases such as Parkinson's disease with gene therapy. Thus, gene therapy for neurodegenerative diseases such as Parkinson's disease was not an unpredictable art. In fact, a number of representative articles are presented below demonstrating that the skilled artisan recognized, and used gene therapy as a means of treating neurodegenerative diseases. The skilled artisan has used a number of different vectors that deliver a particular desired gene, and express the protein in the desired location in the central nervous system. Moreover, the skilled artisan has successfully delivered the vectors to different regions of the brain associated with a neurodegenerative the disease. In fact, the inventors of the present invention also used different vectors to deliver genes to various region of the central nervous system, as evidenced by the following representative articles and abstracts:

(i) Baekelandt *et al.* (2000) *Curr Opin Mol Ther* 2:540-54. This article describes the state of the art at the time the present invention was made in the understanding of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The authors note that gene therapy for Parkinson's disease should be regarded as a realistic alternative to the limited treatment options currently available. This review highlights the most important developments in gene transfer techniques and therapeutic strategies for the central nervous system.

(ii) Bjorklund *et al.* "Towards a neuroprotective gene therapy for Parkinson's disease: use of adenovirus, AAV and lentivirus vectors for gene transfer of GDNF to the nigrostriatal system in the rat Parkinson model." *Brain Res* (2000) 886:82-98. This article reports that during the last few years, recombinant viral vectors derived from *adenovirus (Ad), adeno-associated virus (AAV) and lentivirus (LV)* have been developed into highly effective vehicles for gene transfer to the adult central nervous system. In recent experiments, in the rat model of Parkinson's disease, all three vector systems have been shown to be effective for long-term delivery of glial cell line-derived neurotrophic factor (GDNF) at biologically relevant levels in the *nigrostriatal system.*

Injection of GDNF vectors in the *striatum*, in particular, is effective not only in rescuing the cell bodies in the substantia nigra, but also in preserving the nigrostriatal projection and a functional striatal dopamine innervation in the rat Parkinson model. Long-term experiments using AAV-GDNF and LV-GDNF vectors show, moreover, that sustained GDNF delivery over 3-6 months can promote regeneration and significant functional recovery in both 6-OHDA-lesioned rats and MPTP-lesioned monkeys. The article states that *"The efficacy of the novel AAV and LV vectors in rodent and primate Parkinson models suggests that the time may now be ripe to explore these vector systems as tools for neuroprotective treatments in patients with Parkinson's disease."*

(iii) Bensadoun *et al.* "Lentiviral vectors as a gene delivery system in the mouse midbrain: cellular and behavioral improvements in a 6-OHDA model of Parkinson's disease using GDNF" *Exp Neurol* (2000) 164:15-24. This article reports using *lentiviral vectors* carrying the cDNA for GDNF or mutated GDNF, that were unilaterally injected above the *substantia nigra* of C57BL/6j mice. Two weeks later, the animals were lesioned ipsilaterally with 6-hydroxydopamine into the striatum. Apomorphine-induced rotation was significantly decreased in the GDNF-injected group compared to control animals. Moreover, GDNF efficiently protected 69.5% of the tyrosine hydroxylase-positive cells in the substantia nigra against 6-hydroxydopamine-induced toxicity compared to 33.1% with control mutated GDNF. The article states that *"These data indicate that lentiviral vectors constitute a powerful gene delivery system for the screening of therapeutic molecules in mouse models of Parkinson's disease."*

(iv) Xu *et al.* "Adenovirus-Mediated Gene Therapy of Parkinson's Disease in a Experimental Rat Model." This reference describes the use of *adenovirus* to deliver the tyrosine hydroxylase (TH) gene into the *striatum* of Parkinson's Disease rats. The results showed a significant improvement to the apomorphine-induced rotation movement (approximately 60%). The improvement in rotation movement remained up to 5 months after injection, and TH expression positive cells were found in the vicinity of injection. The article states that *"These results indicate that adenovirus may be a useful carrier for in vivo gene therapy in the PD*

patients."

(v) Leone *et al.* "Multi-site partitioned delivery of human tyrosine hydroxylase gene with phenotypic recovery in Parkinsonian rats." *Neuroreport* (2000) 11:1145-51. This article, that also includes the present inventors as co-authors, reports that the *stereotactic introduction* of a human tyrosine hydroxylase (TH-2) gene using multi-site partitioned doses resulted in behavioral recovery in 6-OHDA-lesioned rats, with transient 100% recovery observed in some animals. The data also show correlation between numbers of TH-immunoreactive cells and loss of apomorphine induced rotation, with a near-linear relationship between TH expression and phenotypic recovery.

(vi) Connor *et al.* "Differential effects of glial cell line-derived neurotrophic factor (GDNF) in the striatum and substantia nigra of the aged Parkinsonian rat" *Gene Ther* (1999) 6:1936-51. This article reports that the injection of an *adenoviral (Ad) vector* encoding human glial cell line-derived neurotrophic factor (GDNF) protects dopaminergic (DA) neurons in the substantia nigra (SN) of young rats. The authors examined whether chronic biosynthesis of GDNF, achieved by adenovirus-mediated delivery of a GDNF gene (AdGDNF), could protect DA neurons and improve DA-dependent behavioral function in aged (20 months) rats with progressive 6-OHDA lesions of the nigrostriatal projection. AdGDNF or control vector was injected unilaterally into either the *striatum or SN.* One week later, rats received a unilateral intrastriatal injection of 6-OHDA on the same side as the vector injection. The results showed that striatal injections of AdGDNF protected against the development of behavioral deficits characteristic of unilateral DA depletion.

(vii) Leff *et al.* "Long-term restoration of striatal L-aromatic amino acid decarboxylase activity using recombinant adeno-associated viral vector gene transfer in a rodent model of Parkinson's disease" *Neuroscience* (1999) 92:185-96. This report show that as a potential treatment for Parkinson's disease, viral vector-mediated over-expression of striatal L-aromatic amino acid decarboxylase was tested in an attempt to facilitate the production of therapeutic

levels of dopamine after peripheral L-dihydroxyphenylalanine administration. Striatal decarboxylation of peripherally administered L-dihydroxyphenylalanine was enhanced by recombinant *adeno-associated virus*-mediated gene transfer of L-aromatic amino acid decarboxylase in unilateral 6-hydroxydopamine-lesioned rats. This gene transfer-induced increase in striatal decarboxylase activity was shown to remain undiminished over a six-month period and transgene expression was demonstrated to persist for at least one year. These data suggest that the use of the non-pathogenic adeno-associated virus to transfer the L-aromatic amino acid decarboxylase gene into the *striatum* of Parkinson's disease patients may be an attractive gene therapy strategy.

(viii) Yamada *et al.* "Herpes simplex virus vector-mediated expression of Bcl-2 prevents 6-hydroxydopamine-induced degeneration of neurons in the substantia nigra in vivo" *Proc Natl Acad Sci U S A* (1999) 96:4078-83. This article describes that injection of the Bcl-2-expressing vector (*herpes simplex virus vector*) into *substantia nigra* (SN) of rats 1 week before injection of 6-OHDA into the ipsilateral striatum increased the survival of neurons in the SN, detected either by retrograde labeling of those cells with fluorogold or by tyrosine hydroxylase immunocytochemistry, by 50%. These results, demonstrating that death of nigral neurons induced by 6-OHDA lesioning may be blocked by the expression of Bcl-2, are consistent with the notion that cell death in this model system is at least in part apoptotic in nature and suggest that a "*herpes simplex virus vector Bcl-2-expressing vector may have therapeutic potential in the treatment of Parkinson's disease.*"

(ix) Choi-Lundberg *et al.* "Behavioral and cellular protection of rat dopaminergic neurons by an adenoviral vector encoding glial cell line-derived neurotrophic factor." *Exp Neurol* (1998) 154:261-75. Previously, the authors had observed that an *adenoviral (Ad) vector* encoding human glial cell line-derived neurotrophic factor (GDNF), injected near the rat *substantia nigra* (SN), protects SN dopaminergic (DA) neuronal soma from 6-hydroxydopamine (6-OHDA)-induced degeneration. In the present study, the effects of Ad GDNF injected into the *striatum*, the site of DA nerve terminals, were assessed. Ad GDNF prevented the development of

behavioral asymmetries which depend on striatal dopamine, including limb use asymmetries during spontaneous movements along vertical surfaces and amphetamine-induced rotation. These studies demonstrate that Ad GDNF can sustain increased levels of biosynthesized GDNF in the terminal region of DA neurons for at least 7 weeks and that this GDNF slows the degeneration of DA neurons and prevents the appearance of dopamine dependent motor asymmetries in a rat model of Parkinson's disease (PD). GDNF gene therapy targeted to the striatum, a more surgically accessible site than the SN, may be clinically applicable to humans with PD.

(x) During *et al.* "Long-term behavioral recovery in parkinsonian rats by an HSV vector expressing tyrosine hydroxylase" *Science* (1994) 266:1399-403. This article by one of the present inventors, shows that one therapeutic approach to treating Parkinson's disease is to convert endogenous striatal cells into levo-3,4-dihydroxyphenylalanine (L-dopa)-producing cells. A defective *herpes simplex virus type 1* vector expressing human tyrosine hydroxylase was delivered into the partially denervated *striatum* of 6-hydroxydopamine-lesioned rats, used as a model of Parkinson's disease. Efficient behavioral and biochemical recovery was maintained for 1 year after gene transfer.

(xi) Kaplitt *et al.* "Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain" *Nat Genet.* (1994) 8:148-54. This article by the present inventors, describes using an *adeno-associated viral (AAV) vector* expressing human tyrosine hydroxylase (AAVth) that was injected into the denervated *striatum* of unilateral 6-hydroxydopamine-lesioned rats (Parkinson's model). Tyrosine hydroxylase (TH) immunoreactivity was detectable in striatal neurons and glia for up to four months and resulted in significant behavioral recovery in lesioned rats treated with AAVth versus AAVlac controls. Safe and stable TH gene transfer into the denervated striatum may have potential for the genetic therapy of Parkinson's disease.

(xii) Kaplitt *et al.* "Viral Vectors for Gene Delivery and Expression in the CNS" *Methods* (1996) 10:343-50. This article by one of the present inventors, describes the use of various viral

vectors for gene expression in the adult mammalian brain. In particular, the defective *herpes simplex virus (HSV)* vector was used to transfer genes into the adult rat brain. This vector was also used for analysis of the preproenkephalin promoter *in vivo*. Finally, the *adeno-associated virus vector* was used to transfer genes into the mammalian brain, and showed significant behavioral recovery in a rodent model of Parkinson's disease.

(xiii) Neve and Geller "A defective herpes simplex virus vector system for gene delivery into the brain: comparison with alternative gene delivery systems and usefulness for gene therapy." *Clin Neurosci.* (1995) 3:262-7. This article describes the use of a defective *herpes simplex virus (HSV)* system for the delivery of exogenous genes into the brain. This system directs precise spatial and temporal expression of recombinant genes in the brain.

(xiv) Geller "Herpesviruses: expression of genes in postmitotic brain cells" *Curr Opin Genet Dev.* (1993) 3:81-5. This article describes gene transfer into neural cells in the adult mammalian brain using vectors derived from the *herpes simplex virus HSV-1* has great promise both for elucidating neuronal physiology and brain mechanisms, and for gene therapy of neurological diseases. Two kinds of HSV-1 vectors were explored: first, defective HSV-1 vectors are small plasmids containing essential HSV-1 cis-acting functions that use HSV-1 mutants as helper virus for packaging; and second, vectors that contain a recombinant gene inserted into the HSV-1 genome. Recently, several genes that alter neuronal physiology have been expressed from defective HSV-1 vectors, both in cultured neurons and *in vivo*.

(xv) Song *et al.* "Modulation of rat rotational behavior by direct gene transfer of constitutively active protein kinase C into nigrostriatal neurons." *J Neurosci.* (1998) 18:4119-32. The modulation of motor behavior by protein kinase C (PKC) signaling pathways in *nigrostriatal neurons* was examined by using a genetic intervention approach. *Herpes simplex virus type 1* (HSV-1) vectors that encode a catalytic domain of rat PKCbetaII (PkcDelta) were developed. PkcDelta exhibited a constitutively active protein kinase activity with a substrate specificity similar to that of rat brain PKC. In the rat brain, microinjection of HSV-1 vectors that

contain the tyrosine hydroxylase promoter targeted expression to *dopaminergic nigrostriatal neurons*. This strategy enabled the demonstration that a PKC pathway or PKC pathways in nigrostriatal neurons modulate apomorphine-induced rotational behavior, and altered dopaminergic transmission from nigrostriatal neurons appears to be the affected neuronal physiology responsible for the change in rotational behavior.

Collectively, these articles demonstrate that the art was replete with teachings that gene therapy was recognized as a realistic alternative treatment for neurodenerative diseases such as Parkinson's disease. Moreover, these articles demonstrate that the a number of *different vectors* had successfully been used to deliver therapeutic genes to *different regions* of the brain. Thus, the level of skill was fairly sophisticated in this arena, and the skilled artisan appreciated that a number of different vectors could be used to deliver a therapeutic gene to different regions of the brain.

In addition to the above cited articles which establish that various vectors can be used to deliver genes to different regions of the central nervous system, there were also a number of articles which identify regions of the brain that required modification. For example, in the field of neurosurgery, the typical treatment for a neurodegenerative disease such as Parkinson's disease was to treat a region of the brain that was overactive, or overstimulated by either deep brain stimulation (DBS) or ablation. Those regions that are overactive, overstimulated, or require some form of modification, in association with a particular neurodegenerative disease, are well known to those skilled in the art (*i.e.*, a neurosurgeon that specializes in neurodegenerative diseases such as Parkinson's disease, epilepsy, Alzheimer's disease, and the like). Thus, the skilled artisan would know exactly which regions of the brain were overactive in Parkinson's disease, and would accordingly take measures to treat such overactive regions. Similarly, a skilled artisan would know exactly which region of the brain required modification for epilepsy, or any other neurodegenerative disease, and take measures to treat those regions. By way of example, typical regions that are routinely treated using surgical procedures for Parkinson's disease by the skilled artisan are identified in the following few articles and

abstracts:

(xvi) Levy *et al.* "Effects of apomorphine on subthalamic nucleus and globus pallidus internus neurons in patients with Parkinson's disease." *J Neurophysiol.* (2001) 86:249-60. This article examines the effect of apomorphine (APO), a nonselective D(1)- and D(2)-dopamine receptor agonist, on the firing activity of neurons in the *subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi)* in patients with Parkinson's disease (PD). Single-unit microelectrode recordings were conducted in 13 patients undergoing implantation of deep brain stimulation electrodes in STN and 6 patients undergoing a pallidotomy. Concurrent with a reduction in limb tremor, the percentage of cells with tremor-related activity (TCs) was found to be significantly reduced from 19 to 6% in the STN and 14 to 0% in the GPi following APO administration.

(xvii) Levy *et al.* "Simultaneous repetitive movements following pallidotomy or subthalamic deep brain stimulation in patients with Parkinson's disease." *Exp Brain Res.* (2002) 147:322-31. This article examines patients with Parkinson's disease (PD) and compares the effects of acute unilateral pallidal lesions (nine patients) and bilateral deep brain stimulation (DBS) of the *subthalamic nucleus (STN)* (eight patients) with levodopa therapy (ten patients) on the performance of isolated versus bilateral simultaneous repetitive movements. The STN group was assessed with and without DBS both on and off levodopa. These observations suggest that the excessive neuronal activity and/or abnormal firing patterns in the globus pallidus internus that is found in parkinsonian patients contribute to difficulties in the execution of complex motor tasks.

(xviii) Dostrovsky *et al.* "Microstimulation-induced inhibition of neuronal firing in human globus pallidus" *J Neurophysiol.* (2000) 84:570-4. Neurosurgical treatment of Parkinson's disease (PD) frequently employs chronic high-frequency deep brain stimulation (DBS) within the internal segment of *globus pallidus* (GPi) and can very effectively reduce L-dopa-induced dyskinesias and bradykinesia, but the mechanisms are unknown. The present study examined the

effects of microstimulation in GPi on the activity of neurons close to the stimulation site. The findings suggest that microstimulation within GPi preferentially excites the axon terminals of striatal and/or external pallidal neurons causing release of GABA and inhibition of GPi neurons.

(xix) Pahapill *et al.* "Tremor arrest with thalamic microinjections of muscimol in patients with essential tremor" *Ann Neurol.* (1999) 46:249-52. Six patients undergoing stereotactic procedures for essential tremor received microinjections of muscimol (a gamma-aminobutyric acid-A [GABA(A)] agonist) into the *ventralis intermedius thalamus* in areas where tremor-synchronous cells were identified electrophysiologically with microelectrode recordings and where tremor reduction occurred with electrical microstimulation. Injections of muscimol but not saline consistently reduced tremor in each patient. The effect had a mean latency of 7 minutes and lasted an average of 9 minutes. Thus, GABA-mediated *thalamic* neuronal inhibition may represent a mechanism underlying the effectiveness of surgery for tremor and that GABA analogues could potentially be used therapeutically.

These articles show that the skilled artisan would know which regions of the brain to target to treat the neurodegeneratiave disease, such as Parkinson's disease, and could also target these regions with a vector carrying the GAD gene to achieve the same result. Thus, based on the knowledge available in the art, one skilled in the art would readily be able to target different regions of the brain with different vectors carrying the GAD gene without undue experimentation.

(B) Teaching in Applicants' specification

Furthermore, in addition to the knowledge available in the art about using different vectors, and targeting different regions, Applicants' own specification provides significant guidance for making and using different vectors to deliver the GAD gene, as well as for delivering a vector carrying the GAD gene to different regions of the brain. Applicant presents several examples that test the *in vivo* effect of GAD expression in Parkinson's disease animal models in accordance with the claimed invention. However, the same methodology and

procedures can readily be applied to treat other neurodegenerative diseases by introducing a vector carrying the GAD gene into regions of the central nervous system that require modification by GAD expression.

(i) *Routine standard molecular biology for making vectors carrying the GAD gene*

Applicants describe in detail how to make and use vectors that carry the GAD gene and how to deliver these vectors to a site in the central nervous system. This is exemplified in the specification with an AAV vector carrying the GAD gene. However, the use of the AAV vectors is merely one example of the numerous vectors that can be constructed to carry the GAD gene using no more than mere routine experimentation. In fact, the specification describes a number of other vectors that can readily used to perform the claimed invention. The specification also provides citations from which the skilled artisan could readily produce a vector carrying a GAD gene using standard molecular biology techniques (*See* page 18, line 31 through page 19, line 11).

> *Alternatively, a vector of the invention can be a virus other than the adeno-associated virus*, or portion thereof, which allows for expression of a nucleic acid molecule introduced into the viral nucleic acid. *For example, replication defective retroviruses, adenoviruses and lentivirus can be used.* Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include Crip, Cre, 2 and Am. The genome of adenovirus can be manipulated such that it encodes and expresses the protein of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. *See e.g.,* Berkner *et al.* (1988) *BioTechniques* 6:616; Rosenfeld *et al.* (1991) *Science* 252:431-434; and Rosenfeld *et al.* (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. (Emphasis added, in part).

The specification also teaches that non-viral vectors can be used (*See* page 19, line 12 through page 20, line 14). Furthermore, in addition to the teaching provided by the application,

articles (i)-(xv) cited above, demonstrate that a number of different vectors were routinely being used to deliver genes to the central nervous system. For example, the adenovirus was used in articles (ii), (iv) and (ix); the lentivirus was used in articles (ii) and (iii); the herpes simplex virus was used in article (viii), (x), (xii), (xiii), (xiv) and (xv); and AAV in articles (ii), (xi), and (xii). Based on the ample guidance in the specification, as well as the knowledge available in the art, a skilled artisan could routinely prepare vectors, other than the AAV vector, to carry a GAD gene without undue experimentation.

(ii) Delivery methods

The specification teaches that the vector can be delivered to regions of the brain that requires modification (*See* page 21, lines 6-7), or is overactive (*See* page 21, line 13) or overstimulated (*See* page 21, line 12) in neurodegenerative diseases. The art is replete with teachings of regions of the brain that are overactive in Parkinson's disease. The articles presented above report that the vectors can be delivered to different regions of the brain such as the striatum (*See e.g.,* (ii), (iv), (vi), (vii), (ix), (x), and (xi)) and the substantia nigra (*See e.g.,* (iii), (vi) and (viii)). Moreover, the skilled artisan could readily identify regions of the brain or central nervous system that require modification to alleviate the symptoms of a neurodegenerative disease. For example, articles (xvi)-(xix) demonstrate surgical procedures that are regularly performed to treat neurodegenerative diseases such as Parkinson's by DBS or ablation by targeting the subthalmic nucleus ((xvi) and (xvii)); the globus pallidus ((xvi) and (xviii)); and the thalamus (xix). These regions can also be targeted for delivery of a vector carrying a GAD gene.

Furthermore, the specification describes in detail how to deliver the vector carrying the GAD gene into specific regions of the brain using stereotactic delivery methods. For example, at page 20, line 24 through page 21, line 14, the specification teaches that:

> Alternatively, precise delivery of the vector into specific sites of the brain, can be conducted using stereotactic microinjection techniques. For example, the

> subject being treated can be placed within a stereotactic frame base (MRI-compatible) and then imaged using high resolution MRI to determine the three-dimensional positioning of the particular region to be treated. The MRI images can then be transferred to a computer having the appropriate stereotactic software, and a number of images are used to determine a target site and trajectory for antibody microinjection. The software translates the trajectory into three-dimensional coordinates that are precisely registered for the stereotactic frame. In the case of intracranial delivery, the skull will be exposed, burr holes will be drilled above the entry site, and the stereotactic apparatus used to position the needle and ensure implantation at a predetermined depth. The vector can be delivered to regions, such as the cells of the spinal cord, brainstem, (medulla, pons, and midbrain), cerebellum, diencephalon (thalamus, hypothalamus), telencephalon (corpus stratium, cerebral cortex, or within the cortex, the occipital, temporal, parietal or frontal lobes), or combinations, thereof. In another preferred embodiment, the vector is delivered using other delivery methods suitable for localized delivery, such as localized permeation of the blood-brain barrier. *Particularly preferred delivery methods are those that deliver the vector to regions of the brain that require modification.*
>
> *Modification as used herein refers to a change in the cellular activity in the region of the brain injected with the vector.* The change in cellular activity can result from changing the expression, or production of genes responsible for stimulating a cell. For example, delivery of a vector comprising a nucleotide sequence encoding GAD, to a region of the brain that is *overstimulated*, such as the basal ganglia. In particular, delivery of the vector to the STN which are *overactive* in diseases such as Parkinson's, will result in expression of GAD in this region. (Emphasis added).

Thus, based on the knowledge available in the art, and the ample guidance in the specification, the skilled artisan could readily deliver a vector carrying the GAD gene to a region of the brain other than the subthalmic nucleus, without undue experimentation.

(C) Applicants' GAD gene therapy procedure approved by the Food and Drug Administration

As further evidence for using gene therapy for the treatment of Parkinson's disease, the Food and Drug Administration has approved gene therapy in a Phase I clinical trial involving 12 patients with Parkinson's disease, for whom current therapies are no longer effective. The clinical study will use the methods of the invention to target overactive cells using the adeno-associated virus carrying the GAD gene. Reports for using gene therapy for the treatment of

Parkinson's disease using the methods of the invention have attained international acclaim, and have been reported throughout the world. A few examples of the numerous world wide reports are presented below:

(xx) Luo *et al* "Subthalmic GAD Gene Therapy In A Parkinson's Disease Rat Model" (2002) *Science* 298: 425-429). This article includes the present inventors and further demonstrates the inventive concept. Using adeno-associated viral vector-mediated somatic cell gene transfer, the authors expressed glutamic acid decarboxylase (GAD), the enzyme that catalyzes synthesis of the neurotransmitter GABA, in excitatory glutamatergic neurons of the STN in rats. The transduced neurons, when driven by electrical stimulation, produced mixed inhibitory responses associated with GABA release. This phenotypic shift resulted in strong neuroprotection of nigral dopamine neurons and rescue of the parkinsonian behavioral phenotype. This strategy suggests that there is plasticity between excitatory and inhibitory neurotransmission in the mammalian brain that could be exploited for therapeutic benefit.

(xxi) Cornell Chronicle October 17, 2002
(http://www.news.cornell.edu/chronicle/02/10.17.02/Parkinsons-Weill.html)

Based on the methods of the invention, and other data, the *U.S. Food and Drug Administration (FDA) has given its approval to begin testing the therapy in a Phase I clinical trial.*

(xxii) Gene therapy for Parkinson's BBC News
(http://news.bbc.co.uk/1/hi/health/2316613.stm)
Doctors are to begin the first ever trial using gene therapy to treat Parkinson's Disease.

(D) The references cited in the Office Action are dated, but also support Applicants' position.

The Office Action relies on a number of *dated references* that negatively portray gene therapy. However, there have been several advances in this area of research which have resulted

in better vectors for delivery of the gene, better procedures of targeting the desired cells, and better control of gene expression. Thus, gene therapy is an evolving field, and there have been many advances in the procedure that indicate that diseases involving the central nervous system, especially, the brain, are particularly conducive to this form of therapy. The brain and central nervous system is immunocompromised, therefore, there is less of a chance of developing the an immune response against the virus. In fact, at the time of filing the application, a number of studies had successfully been conducted for using a variety of different vectors that had been delivered to target regions in the brain. In many of these studies, long term expression of the protein occurred, and resulted in a therapeutic effect in the animal (*See* articles (i)-(xv) above).

However, even the dated references cited in the Office Action recognize the benefits of gene therapy for the brain. For example, the benefits are recognized in Verma *et al* (1997), who reports that:

> "In the *brain* however, gene *transfer to just a few hundred cells could considerably benefit patients with neurological diseases*" (*See* page 239, end of column 2 and the beginning of column 3).

The reference further reports that:

> "lentivirus injected into rodent brains...give sustained expression for over six months" Also, that "injection of 10^7 infectious units *does not elicit the cellular immune response* at the site of injection. . .So, at present, lentiviral vectors seem to offer an excellent opportunity for in vivo gene delivery with sustained expression." (*See* page 240, column 3 and page 241, column 1).

With regard to Crystal (1995), this reference reflects the state of the art at that time (*i.e.*, in 1995). While issues relating to the use of vectors and gene transfer may have posed a problem in 1995, there have been significant advances since then, particularly in the field of gene therapy for the brain such as Parkinson's disease. This is demonstrated by the representative number of articles that were in existence at the time the application was filed (*See* articles (i)–(xv) above). These articles show that a large number of different vectors could clearly be used to deliver

different therapeutic genes to regions of the brain, express the gene, and show some therapeutic effect Parkinson's subjects. Furthermore, even Crystal (1995) recognizes that "none of the drug development problems facing human gene transfer are *insumountable*, but each will take time to solve." It appears that the some of the problems of using gene therapy for Parkinson's disease were already solved, or in the process of being solved, at the time the application was filed.

With regard to Friedman (1997), the article reports at page 101, last paragraph, that:

> "although I have dwelled on certain technical challenges to gene therapy, I am nonetheless *highly optimistic* that it will soon begin to prove helpful for some disease. Our tools are improving rapidly, and some of the burgeoning clinical trials clearly are on the verge of demonstrating real merit in ameliorating disease, even with today's imperfect techniques." (Emphasis added).

Thus, even Friedman recognizes that the "tool are improving rapidly" and the author is "highly optimistic that it will soon begin to prove helpful for some disease" Parkinson's disease appears to be such a disease for which the tools have improved, and is recognized by those skilled in the art as a filed that could benefit enormously from gene therapy at the time the application was filed.

With regard to Miller (1995), this article describes various vectors and their uses in gene therapy "*which are currently only at the experimental level*". At the time this reference was published, the field of gene therapy was fairly young. Since then, and at the time the present application was filed, there have been considerable advances in the arena of gene therapy of the brain, as evidenced by the representative number of references cited above.

With regard to the NIH ad hoc committee report that assesses the current status and promise of gene therapy reported in December *1995*. This states that "clinical efficacy has not been definitively demonstrated at this time in any gene therapy protocol, despite anecdotal claims" and that "significant problems remain in all basic aspects of gene therapy" (Orkin and

Motulsky, page 1).

Since this report, there have been several advancements in the arena of gene therapy, particularly in the area of the brain. Furthermore, there is an ongoing interest in gene therapy, as evidenced by the (i) NIH News release entitled "New Initiatives to Protect in Gene Therapy"– HHS Press Release of 03/07/2000; and (ii) New Initiatives to Protect Participants in Gene Therapy Trials" 03/07/2000, in which the FDA and the NIH announced two new initiatives to strengthen the safeguards for individual enrolled in clinical studies for gene therapy.

Furthermore, the recent FDA approval of the small scale clinical study on Parkinson's disease patients using the methods of the invention demonstrate that there is a continued interest in gene therapy, particularly for gene therapy of the brain. It also demonstrates that administrative agencies recognize the feasibility of Applicants' invention, to the extent that they have approved trials in Parkinson's patients.

For all the forgoing reasons, the claimed invention could readily be performed using vectors other than AAV, and targeting regions other than the subthalmic nucleus, without undue experimentation. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

Rejection of Claims 12-19 Under 35 U.S.C. §112 Second Paragraph

Claims 12-19 have been rejected under 35 U.S.C. § 112, second paragraph as "being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention". In particular, "Claims 12-19 are indefinite in their recitation of 'expressing the GAD in the region of the brain an amount effective' because it appears that the phrase should read 'in an amount effective.'"

Applicants thank the Examiner for pointing out this error, and have amended the claims as suggested by the Examiner.

New Claims

New independent claim 26 and dependent claims 27-31 are presented. The principal claim 26 recites a method for treating Parkinson's disease by identifying one or more regions of the brain that require modification; delivering an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding a glutamic acid decarboxylase (GAD) to the region of the brain; and expressing the GAD in the region of the brain in an amount effective to treat or reduce Parkinson's disease.

Drawings

Applicant submits herewith new formal drawings, as requested.

Conclusion

For all the reasons above, reconsideration and allowance are requested. The Examiner is urged to call the undersigned at the telephone number indicated below so that any remaining issues can be discussed.

Respectfully submitted,
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Marked-up version of all pending claims

1. (Original) A method for treating a neurodegenerative disease in a subject comprising:

identifying a target site in the central nervous system that requires modification;

delivering a vector comprising a nucleotide sequence encoding a glutamic acid decarboxylase (GAD) to the target site in the central nervous system; and

expressing the GAD in the target site in an amount effective to treat or reduce the neurodegenerative disease.

2. (Original) The method of claim 1, wherein the vector is a viral vector.

3. (Original) The method of claim 2, wherein the a viral vector is selected from the group consisting of adenovirus vectors, herpes virus vectors, parvovirus vectors, and lentivirus vectors.

4. (Original) The method of claim 2, wherein the a viral vector is an adeno-associated viral vector.

5. (Original) The method of claim 1, wherein the vector is a non-viral vector.

6. (Original) The method of claim 5, wherein the non-viral vector is a liposome-mediated delivery vector.

7. (Original) The method of claim 1, wherein the vector is delivered using stereotaxic delivery.

8. (Original) The method of claim 1, wherein the target site in the central nervous system is a region of the brain.

9. (Original) The method of claim 8, wherein the region of the brain is selected from the group consisting of basal ganglia, subthalmic nucleus (STN), pedunculopontine nucleus (PPN), substantia nigra (SN), thalmus, hippocampus, cortex, and combinations thereof.

10. (Original) The method of claim 8, wherein the region of brain is the subthalmic nucleus (STN).

11. (Cancel) The method of claim 1, wherein the neurodegenerative disease is selected from the group consisting of Parkinson's disease, Alzheimer's disease, senile dementia, Amyloid Lateral Schlerosis (ALS), and epilepsy.

12. (Amended) A method for treating Parkinson's disease in a subject comprising:

identifying one or more regions of the brain that require modification;

delivering a vector comprising a nucleotide sequence encoding a glutamic acid decarboxylase (GAD) to the region of the brain; and

expressing the GAD in the region of the brain in an amount effective to treat or reduce Parkinson's disease.

13. (Original) The method of claim 12, wherein the vector is a viral vector.

14. (Original) The method of claim 13, wherein the a viral vector is selected form the group consisting of adenovirus vectors, herpes virus vectors, parvovirus vectors, and lentivirus vectors.

15. (Original) The method of claim 13, wherein the a viral vector is an adeno-associated viral vector.

16. (Original) The method of claim 12, wherein the vector is a non-viral vector.

17. (Original) The method of claim 16, wherein the non-viral vector is a liposome-mediated delivery vector.

18. (Original) The method of claim 12, wherein the region of the brain is selected from the group consisting of basal ganglia, subthalmic nucleus (STN), pedunculopontine nucleus (PPN), substantia nigra (SN), thalmus, hippocampus, cortex, and combinations thereof.

19. (Original) The method of claim 12, wherein the region of brain is the subthalmic nucleus (STN).

26. (New) A method for treating Parkinson's disease in a subject comprising:

identifying one or more regions of the brain that require modification;

delivering an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding a glutamic acid decarboxylase (GAD) to the region of the brain; and

expressing the GAD in the region of the brain in an amount effective to treat or reduce Parkinson's disease.

27. (New) The method of claim 26, wherein the adeno-associated viral vector is selected from the group consisting of AAV-1 AAV-2, AAV-3, AAV-4, AAV-5 and AAV-7.

28. (New) The method of claim 26, wherein the adeno-associated viral vector is AAV-2.

29. (New) The method of claim 26, wherein the region of the brain is selected from the group consisting of basal ganglia, subthalmic nucleus (STN), pedunculopontine nucleus (PPN), substantia nigra (SN), thalmus, hippocampus, cortex, and combinations thereof.

30. (New) The method of claim 26, wherein the region of brain is the subthalmic nucleus (STN).

31. (New) The method of claim 26, wherein the region of brain is the substantia nigra (SN).